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RESEARCH ARTICLE

Inbreeding in reintroduced populations: the effects of early reintroduction history and contemporary processes

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Abstract Maintaining genetic variation and minimizing inbreeding are central goals of conservation genetics. It is therefore crucial to understand the important population parameters that affect inbreeding, particularly in reintroduction programs. Using data from 41 reintroduced Alpine ibex (*Capra ibex ibex*) populations we estimated inbreeding since the beginning of reintroductions using population-specific Fst, and inbreeding over the last few generations with contemporary effective population sizes. Total levels of inbreeding since reintroduction of ibex were, on average, close to that from one generation of half-sib mating. Contemporary effective population sizes did not reflect total inbreeding since reintroduction, but 16% of variation in contemporary effective population sizes among populations was due to variation in current population sizes. Substantial variation in inbreeding levels among populations was explained by founder group sizes and the harmonic mean population sizes since founding. This study emphasizes that, in addition to founder group sizes, early population growth rates are important parameters determining inbreeding levels in reintroduced populations.

Keywords Alpine ibex · Effective population size · Fst · Linkage disequilibrium · Population specific inbreeding · Reintroductions

Introduction

Maintaining genetic variation and minimizing inbreeding are among the central goals of conservation genetics (e.g. Hedrick and Miller 1992). In populations that are subject to conservation management, some population parameters that may impact inbreeding and the loss of genetic variation may be under (partial) control of the managers. For example, in reintroduction programs managers may have control over the timing and the number of founders released, two parameters that will affect the degree of inbreeding in the resulting populations (Allendorf and Luikart 2007). Thus, identifying the population parameters that contribute most to inbreeding and the loss of genetic variation is an important task for conservation biologists (Frankham et al. 2002; Allendorf and Luikart 2007).

In an idealized Wright–Fisher population inbreeding accumulates at a constant rate (Crow and Kimura 1970, p. 101). However, natural populations are not ideal: they change in size, in sex ratio and in variance of reproductive success. Therefore, the accumulation of inbreeding is not constant over time (Chesser et al. 1993; Wang 2005). Particularly the small population sizes following a bottleneck or founder event are a key factor in contributing to inbreeding, because inbreeding increases per generation proportionally to the reciprocal of the population size (Crow and Kimura 1970, p. 101). Even a population that has recovered from a bottleneck will experience further inbreeding each generation due to its finite population size. This inbreeding due to finite population size after recovery from a bottleneck may be non-trivial and adds each generation to the already existing inbreeding that accumulated through the past population history (e.g. Ewing et al. 2008). In this study, we aimed to decompose inbreeding into the contributions of different periods of the reintroduction history and to identify those population parameters that have influenced levels of inbreeding the most.

All inbreeding estimates are relative and there are several estimators of inbreeding that differ in their reference

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populations (Jacquard 1975; Keller and Waller 2002). Different reference populations correspond to different time periods over which inbreeding has accumulated and they can therefore be used to decompose inbreeding levels of populations into the contributions from different time periods in the past (Jacquard 1974, p. 169). If inbreeding occurs both due to subdivision in finite populations and non-random mating within subpopulations, overall inbreeding is given by $(1 - \text{Fit}) = (1 - \text{Fis})(1 - \text{Fst})$ (Wright 1969, p. 295). Thus, $\text{Fis} = 0$ does not imply an absence of inbreeding as measured by Fit. If Fis is zero, mating in the subpopulations is random and overall inbreeding equals Wright's Fst (Keller and Waller 2002).

The rate of inbreeding in a population depends on the effective population size (Ne), which refers to the size of an idealized Wright–Fisher population experiencing the same rate of inbreeding as the real population under study (Crow and Kimura 1970, p. 103). The Ne of a population can be determined by life history data, where available. Without detailed life history information, Ne estimated from demographic data might be inaccurate (Frankham 1995), because not all relevant factors (population size and its fluctuations, variance in reproductive success and sex ratio; Nunney 1991, 1993; Frankham 1995) will be taken into account. In contrast to demographic data, genetic data can provide Ne estimates that include all factors that influence effective population size, but the resulting estimates have the disadvantage of large confidence intervals especially for larger effective population sizes (Nei and Tajima 1981; Waples 1989). Nevertheless, genetic data are often the only practical way to estimate effective population sizes of wild populations.

Here we estimate effective population sizes and inbreeding in 41 populations of Alpine ibex (*Capra ibex ibex*) from genetic data. Alpine ibex were successfully reintroduced to the European Alps starting in 1911 (Grodinsky and Stuwe 1987; Stuwe and Scribner 1989; Maudet et al. 2004; Biebach and Keller, 2009) with many populations descending from one common ancestral population in northern Italy. Reintroduction histories are well known for many populations (Biebach and Keller 2009). This allowed us to decompose total inbreeding into contributions from various phases of the reintroduction and to relate these inbreeding estimates to demographic parameters such as founder group size, current census population size and effective population size.

Materials and methods

Populations and samples

Between 2004 and 2007 we collected 1206 tissue and blood samples from both sexes and all age classes from 41 wild Alpine ibex populations across Switzerland. Average sample size per population was 29 (range: 18–61; Table 1). For detailed information on the populations and sampling see Biebach and Keller (2009). Note that Biebach and Keller (2009) studied one additional population (the ancestral Italian population), which we did not include here because that population is not descendent from the Swiss zoo populations. Swiss hunting authorities provided data on current census sizes in 2007 (Nc) for all populations and yearly census sizes over the last 30–40 years for 26 populations.

For 10 of the 41 wild populations we additionally had 258 tissue samples collected between 1986 and 1988 (Stuwe and Scribner 1989; Table 1). Thus, assuming a generation time of 8 years in Alpine ibex (Grodinsky and Stuwe 1987) we had samples for these populations from two sampling periods two to three generations apart. We could exclude the possibility of sampling an individual in both time periods, because tissue samples of the first sampling period (1986–1988) were solely collected from legally hunted animals.

Genetic data

All samples were genotyped at 37 neutral microsatellites as described in Biebach and Keller (2009). Unreliable genotypes were repeated up to three times and only reliable genotypes were used for further analysis. Genotypes were classified as unreliable, if the electropherogram departed from the characteristic pattern for a given locus. We estimated allelic dropout and false allele rates for the first sampling period (1986–1988) by repeating between 9.3 and 46.2% of the samples for individual loci. We used a maximum-likelihood-based method implemented in PEDANT (Johnson and Haydon 2007) to estimate genotyping error rates, as we had done previously for the 2004–2007 sampling period (Biebach and Keller 2009).

Contemporary effective population size

We estimated Ne using two methods: allele frequency changes through time (temporal method) and linkage disequilibrium at a single point in time (LD method). We used both methods because they make different assumptions and thus provide somewhat complementary answers. Both methods assume isolated populations without immigration, a reasonable assumption for most ibex populations as they live on mountain tops separated by geographic structures that lead to no or low gene flow between populations (Maudet et al. 2004; Biebach and Keller 2009).

The temporal method uses the fact that in the absence of other forces such as migration, selection and mutation allele frequency changes over time are solely a function of genetic drift and can be used to estimate variance Ne (Wang 2001).

Table 1 Population parameters for 41 Alpine ibex populations

Population	Sample size 2004–2007	Sample size 1986–1988	Census size 2007	Temporal Ne (95% CI)	LD Ne (95% CI)	Total inbreeding (95% CI)	Split inbreeding (95% CI)
Adula-Vial	37	NA	297	NA	298 (50, 391)	0.08 (0.05, 0.11)	NA
Albris	61	23	1033	50 (25, 155)	84 (55, 145)	0.09 (0.06, 0.12)	0.01 (0, 0.02)
Aletsch-Bietschhorn	43	NA	531	NA	75 (42, 82)	0.06 (0.04, 0.08)	NA
Alpstein	30	22	148	651 (45, ∞)	54 (33.5, 310)	0.18 (0.13, 0.23)	NA
Arolla	36	NA	343	NA	27 (24, 44)	0.09 (0.06, 0.12)	0.04 (0.02, 0.06)
Bire-Oeschinen	18	27	68	86 (28, ∞)	24 (12, 28)	0.12 (0.08, 0.16)	0.04 (0.01, 0.07)
Brienzer-Rothorn	39	26	157	61 (27, 399)	81 (56, 298)	0.11 (0.08, 0.15)	0.04 (0.01, 0.06)
Calanda	31	NA	131	NA	26 (17, 35)	0.17 (0.12, 0.23)	0.08 (0.05, 0.12)
Cape au Moine	49	NA	238	NA	120 (61, 207)	0.13 (0.1, 0.16)	NA
Churfirsten	24	NA	168	NA	19 (15, 31)	0.09 (0.06, 0.12)	NA
Crap da Flem	27	NA	103	NA	27 (20, 47)	0.13 (0.09, 0.18)	0.08 (0.05, 0.12)
Dents du Midi	23	NA	189	NA	85 (46, ∞)	0.11 (0.08, 0.15)	0.05 (0.02, 0.07)
Ferret	19	NA	180	NA	10 (9, 16)	0.09 (0.06, 0.13)	NA
Fluebrig	32	NA	208	NA	51 (37, 142)	0.16 (0.12, 0.21)	NA
Flueela	21	NA	340	NA	181 (42, ∞)	0.14 (0.1, 0.2)	0.04 (0.02, 0.07)
Foostock	27	NA	330	NA	65 (40, ∞)	0.13 (0.1, 0.19)	NA
Gornergrat	23	NA	117	NA	19 (19, 42)	0.1 (0.07, 0.14)	NA
Graue Hoerner	47	31	285	108 (40, ∞)	50 (36, 74)	0.1 (0.07, 0.13)	NA
Gross Lohner	22	NA	150	NA	254 (79, ∞)	0.03 (0.02, 0.05)	NA
Hochwang	28	NA	117	NA	47 (33, 128)	0.14 (0.1, 0.19)	0.07 (0.05, 0.11)
Julier Nord	19	NA	486	NA	173 (34, ∞)	0.14 (0.1, 0.2)	0.05 (0.03, 0.09)
Julier Sued	23	25	312	22 (14, 38)	55 (31, 225)	0.1 (0.07, 0.15)	0.03 (0.01, 0.05)
Justistal	19	NA	80	NA	98 (47, ∞)	0.13 (0.09, 0.17)	0.08 (0.04, 0.12)
Macun	22	NA	139	NA	28 (19, 46)	0.12 (0.08, 0.17)	0.05 (0.03, 0.08)
Mischabel	33	15	597	NA	355 (86, ∞)	0.13 (0.09, 0.17)	0.06 (0.04, 0.09)
Muveran	27	NA	387	NA	37 (27, 71)	0.1 (0.07, 0.14)	0.07 (0.04, 0.1)
Nufenen	19	NA	103	NA	70 (35, ∞)	0.1 (0.07, 0.14)	NA
Oberbauestock	30	NA	200	NA	20 (18, 46)	0.2 (0.15, 0.27)	NA
Pierreuse-Gummfluh	41	NA	157	NA	72 (53, 134)	0.07 (0.05, 0.09)	NA
Pilatus	17	NA	104	NA	18 (9, 23)	0.16 (0.11, 0.21)	0.08 (0.05, 0.12)
Pleureur	23	47	590	NA	877 (131, ∞)	0.08 (0.05, 0.11)	0.01 (0, 0.02)
Rheinwald	35	NA	417	NA	48 (38, 119)	0.12 (0.09, 0.16)	0.04 (0.03, 0.06)
Rothorn-Weissfluh	29	NA	316	NA	101 (60, ∞)	0.1 (0.07, 0.14)	0.02 (0.01, 0.04)
Schwarzmoench	32	17	177	58 (25, 719)	61 (40, 102)	0.06 (0.04, 0.09)	NA
Tanay	25	NA	300	NA	27 (22, 54)	0.15 (0.11, 0.2)	0.08 (0.06, 0.12)
Umbrail	29	NA	84	NA	22 (15, 28)	0.11 (0.08, 0.15)	0.07 (0.04, 0.1)
Val Bever	32	NA	213	NA	NA	0.09 (0.06, 0.13)	0.01 (0, 0.03)
Weisshorn	25	NA	354	NA	NA	0.13 (0.1, 0.17)	0.08 (0.05, 0.11)
Weissmies	49	NA	412	NA	215 (89, ∞)	0.1 (0.07, 0.13)	NA
Wetterhorn	19	NA	70	NA	15 (11, 22)	0.18 (0.13, 0.23)	NA
Wittenberg	21	25	126	43 (21, 175)	87 (39, 535)	0.06 (0.04, 0.08)	NA

The temporal method estimates the harmonic mean Ne for the time between the two temporal samples. There are several statistical approaches to estimate Ne from temporal samples that tend to give similar results in comparative studies (Aspi et al. 2006; Fraser et al. 2007). Thus, we used only one temporal method, a Bayesian coalescent-based method implemented in CONE (Anderson 2005). We set the generation time between the two sampling periods to 2, calculated the likelihood for Ne ranging from 2 to 800 in steps of 2 and used 1000 Monte Carlo replications. We applied this method to the ten populations with samples from the two sampling periods.

The LD method estimates Ne in a single sample from LD at neutral loci generated from genetic drift in isolated populations with random mating (Hill 1981). In populations of constant size the LD method gives the Ne of the parental generation (Waples 2005). In growing or declining populations LD is influenced by the last few generations, because it takes *ca.* 3–5 generations to reach a new asymptotic LD (Waples 2005). Therefore, in a species such as ibex where population sizes are not constant, the LD method reflects the harmonic Ne of the last few generations (Waples 2005). We assumed that the last four generations influenced LD Ne in ibex. LD is biased downwardly if alleles at low frequencies are included in the samples (Hudson 1985). Simulations show that LD is not biased if allele frequencies below 0.05 are excluded (Hudson 1985; Waples and Do 2008) and hence we used only allele frequencies above 0.05 for the LD analysis. We used LDNE (Waples and Do 2008) to estimate Ne for a random mating system (Fis ≈ 0 in ibex populations; Biebach and Keller 2009). Confidence intervals were estimated by the jackknife method (Waples and Do 2008). The LD method has the advantage of requiring only one sampling time and thus we were able to estimate LD Ne for all 41 populations of the 2004–2007 sampling period.

Due to an extreme outlier (Fig. 2) we used a Spearman rank correlation to compare the temporal and LD Ne estimators. We calculated the ratio of the two estimators to investigate any systematic difference between the two. We used LD Ne for further analysis, because we had data from all 41 populations. For comparison with Ne, we calculated the harmonic mean census population sizes over the last four generations for those 26 populations where data were available. We used the harmonic mean, because the effect of fluctuating population size on Ne is given by the harmonic mean population size (Crow and Kimura 1970, p. 360). Harmonic mean census sizes over the last four generations correlated highly ($r = 0.97$, $P < 0.001$) with census sizes (Nc) of these 26 populations in 2007. While highly correlated, the harmonic mean population size over the last four generations was on average 15% below the census size 2007. This difference has no effect on the slope of regression analyses, but it is critical for the ratio Ne/Nc. Therefore, to use data for all 41 populations whenever possible, we used census data of the year 2007 for a regression of Ne on Nc, but the harmonic mean census size over the last four generations of the 26 populations to calculate the Ne/Nc ratio.

Inbreeding

To quantify inbreeding that accumulated since the establishment of the reintroduced ibex populations we estimated population-specific Fst. Vitalis et al. (2001, Eq. 8) define this population-specific Fst. In Alpine ibex, population-specific Fst quantifies total inbreeding that arose due to drift relative to the last common ancestral population because there is no inbreeding due to non-random mating (Fis is not significantly different from zero in any population; Biebach and Keller 2009).

We estimated population-specific Fst from the microsatellite data using a likelihood approach and the software 2MOD (Ciofi et al. 1999). 2MOD uses coalescent theory and Markov chain Monte Carlo (MCMC) simulations to calculate the relative likelihood of two demographic models, an equilibrium drift-migration model and a non-equilibrium drift model, given the allele frequencies of the populations. Initial analyses of our data revealed no support for the gene-flow model (support for gene-flow model was 0% from 450 000 iterations; Biebach and Keller 2009). Therefore, using a slightly modified version of 2MOD we fixed the analysis to the non-equilibrium drift model to estimate inbreeding relative to the last common ancestral population. The model assumes that the reciprocal of the mutation rate is much longer than the divergence time (Ciofi et al. 1999), which is a reasonable assumption for the Swiss ibex populations since they were founded no more than 12 generations ago.

To decompose total inbreeding into contributions from different phases of the reintroduction, we estimated population-specific Fst over two time spans, once over the whole reintroduction period since the first releases from the zoo populations (total inbreeding) and once since the last split when many populations were founded several generations after the first populations were established (split inbreeding) (Fig. 1). Fst estimates over the whole time

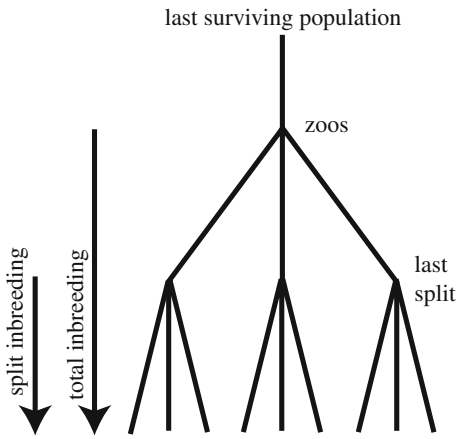

Fig. 1 Schematic diagram of the reintroduction history of Alpine ibex. Population-specific Fst was estimated over two time periods: since the zoo populations (total inbreeding) and since the last split of the populations (split inbreeding). Last split refers to a time period when many populations were founded several generations after the first populations were established

span quantify inbreeding for all 41 Swiss populations relative to the zoo populations approximately 12 generations ago. These estimates of total inbreeding represent inbreeding that accumulated over one (e.g. Albris, Brienzer-Rothorn and Pleureur) and two founder events (those founded at the last split), respectively (Fig. 1). We estimated inbreeding relative to the ancestral population at the last split (split inbreeding) for 20 populations that were founded from one of three wild source populations four to six generations ago. Each of the three sources and its descendant populations was analysed separately in 2MOD. Split inbreeding estimates inbreeding that accumulated since the second founder event for those populations that experienced two founder events. Split inbreeding was also calculated for the three populations that were the sources (Albris, Brienzer-Rothorn and Pleureur) of the populations founded at the last split. For these three source populations, split inbreeding quantifies the inbreeding that accumulated over the same time as the other populations in the split inbreeding analysis but without an additional founder event (Table 1).

We explored the effects of harmonic mean population size and of founder group size on split inbreeding. The harmonic mean of the effective population sizes (Crow and Kimura 1970, p. 360) is expected to determine the amount of inbreeding over T generations as follows: (Crow and Kimura 1970, p. 331)

$$\mathrm{Fst} = 1 - e^{-T/2\mathrm{Ne}} \quad (1)$$

To linearize the expected relationship between population-specific Fst calculated from molecular markers and the harmonic mean population sizes calculated from census data, we used Eq. 1 to convert the harmonic mean census population sizes into Fst (hm Fst). We omitted the first 5 years after the initial releases in the calculations of hm Fst to avoid biases due to missing data (which were common in the first years after a release) and different release modes. For example, the harmonic mean would differ if all individuals were released in the first year or over two consecutive years, though the inbreeding and variance effective population size would be similar under the reasonable assumption of no reproduction in the first year. For the three source populations of the last split, we used the census population size in the mean founding year (1961) to calculate founder group size, and census data afterwards to calculate hm Fst. Founder group size and harmonic mean population size are expected to correlate since a low founder group size leads to a low harmonic mean population size. Thus, founder group size can have a direct effect on inbreeding and an indirect one mediated via the harmonic mean census size. We used path analysis (Mitchell 1993) and PROC TCALIS in SAS to disentangle these two effects.

With the estimates of total inbreeding and split inbreeding we were able to decompose total inbreeding into the inbreeding that accumulated up to the last split (inbreeding before last split) and since the last split (split inbreeding). These two time periods correspond to approximately the first and last six generations of the time since the initial reintroductions. Inbreeding that accumulated before the last split was calculated from total and split inbreeding using the relationship: (Jacquard 1974, p. 169)

$$(1 - \mathrm{Fst.total}) = (1 - \mathrm{Fst.1st})\,(1 - \mathrm{Fst.2nd}) \quad (2)$$

where Fst.total is total inbreeding, Fst.1st is inbreeding up to the last split and Fst.2nd is split inbreeding.

Unless stated otherwise, all statistical analyses were performed in the software package R, version 2.8.0 (R Development Core Team 2006).

Results

Temporal genetic data

Across all 37 microsatellite loci, the allelic dropout rate for the 1986–1988 sampling period ranged from zero to 23.8% per heterozygote (mean: 3.4%) and the false allele rate per genotype ranged from zero to 5.3% (mean: 0.5%; Appendix Table 2). Six markers had dropout rates above 5% and three of these had false allele rates above 1%. Only one marker, BM1225, had a false allele rate above 1% but no dropout errors. This locus and loci with dropout error rates above 5% (BM1225, BM4505, HAUT27, INRABERN175, INRABERN185, SR-CRSP08, URB058; Appendix 1) were omitted from both sampling periods (1986–1988 and 2004–2007) for Ne estimation with the temporal method. Genotyping error rates were much lower in the 2004–2007 samples (generally below 1%; Biebach and Keller 2009) and thus all loci were retained in the analyses using only those samples.

Effective population sizes

Valid estimates of the effective population size with the temporal method were obtained for eight of the ten populations (Table 1). For two populations (Mischabel and Pleureur) no estimate was obtained within the pre-defined range (2–800) even though their census sizes were approximately 600. There was very little genetic drift between the two sampling periods in these two populations (pairwise Fst between the two sampling periods: −0.006 and 0.0113, respectively) suggesting that random sampling explains the lack of convergence (manual of CONE, Anderson 2005). For the remaining eight populations the temporal maximum likelihood estimates of Ne ranged

between 22 and 651 with a mean of 135. The three highest estimates had infinite upper confidence intervals (Table 1), which is in line with the expectation that precision is poorer if Ne increases because in larger populations genetic drift decreases and the signal of genetic drift may be overridden by the sampling error (Waples 1989).

Two populations (Val Bever and Weisshorn) also failed to yield estimates of the effective population size using the linkage disequilibrium method, but these were not the same populations that failed to give estimates with the temporal method (Table 1). The two populations yielded negative Ne estimates because LD due to sampling error was higher in these two populations than LD due to genetic drift (Bartley et al. 1992). In the other populations, the LD method yielded effective population size estimates from 10 to 877 with a mean of 102. Eleven estimates had infinite upper 95% confidence intervals. 17 of the 39 LD Ne estimates were below 50 and 12 of these had also upper confidence intervals below 50. LD Ne estimates of three populations (Adula Vial, Gross Lohner and Pleureur) were above the census size, but the lower confidence intervals included the census population size.

The two methods of estimating effective population size did not yield similar results: Although confidence intervals of the two methods overlapped for all except two populations (Julier Sued and Bire Oeschinen), the two sets of estimates were not positively correlated (Fig. 2; $r = -0.64$, $P = 0.1$, $n = 8$). At the same time, there seemed to be no systematic difference between the two methods: The mean ratio of LD Ne to temporal Ne was 1.18 (95% CI: −0.52, 2.88). In the following we used the LD Ne estimates because we had estimates from 39 populations instead of eight obtained with the temporal method.

Census size in 2007 (Nc) had a significant effect on contemporary effective population size (Fig. 3; $b = 0.33 \pm 0.12$ SE, $F = 8.3$, $R^2 = 0.16$, $n = 39$, $P = 0.007$). Two populations, Albris and Pleureur represented outliers in this regression, but removing them from the analysis did not substantially alter the results. However, Nc explained only 16% of the variation in Ne (24% with the two outliers excluded). The mean ratio between the Ne estimates and the harmonic mean census sizes over the last four generations was 0.58. When unrealistic values, where Ne exceeded Nc, were removed, the mean ratio was 0.34 (range: 0.1–0.75).

Inbreeding during the two phases of the reintroduction program

Mean total inbreeding relative to the zoo populations (Fig. 1) was 0.11 (SD ± 0.04), but inbreeding varied greatly (up to 84%) among populations (Table 1). Total inbreeding (Fst) estimated with the likelihood approach in 2MOD was only moderately ($r = 0.7$) correlated with the

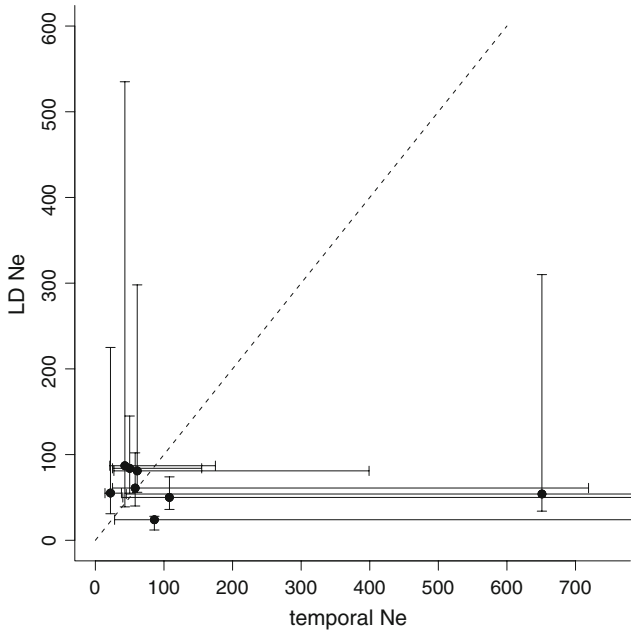

Fig. 2 Estimates of contemporary effective population sizes and 95% confidence intervals obtained with the temporal method (temporal Ne) and linkage disequilibrium method (LD Ne). The *dotted line* represents the 1:1 ratio

Weir and Cockerham type estimator (Weir and Cockerham 1984) of Fst (data not shown). Such differences between moment based and maximum likelihood based Fst estimators are commonly observed, but poorly understood (Beaumont, "personal communication"). LD Ne had no effect on total inbreeding ($b = -6.8 \times 10^{-05} \pm 3.9 \times 10^{-05}$ SE, $F = 3.1$, $R^2 = 0.05$, $n = 39$, $P = 0.09$).

Mean split inbreeding was 0.051 (sd ± 0.025), i.e. approximately half as high as total inbreeding, and varied similarly among populations (92%; Fig. 6). Harmonic mean census size (transformed to hm Fst) and founder group size (ln transformed) each had a significant effect on inbreeding since the last split (linear regressions, $b = 0.95 \pm 0.27$ SE, $F = 12.2, R^2 = 0.48, n = 13, P = 0.005$ and $b = -0.02 \pm 0.01$ SE, $F = 12.7$, $R^2 = 0.49$, $n = 13$, $P = 0.004$; Fig. 4). Path analysis revealed that founder group size had both a direct effect on inbreeding and an indirect effect mediated by harmonic mean census size (approximately half as strong as the direct effect; Fig. 5). Overall, founder group size and harmonic mean census size explained 64.6% of the variation in inbreeding in the path analysis.

Mean inbreeding before the last split was 41% higher than that after the last split (0.072 ± SD 0.016 vs. 0.051 ± SD 0.025). The ratio of inbreeding before the last split to inbreeding after the last split averaged 2.4, with a range between 0.6 and 10.3. However, if we consider only the populations that experienced two founder events the ratio averaged only 1.7 (range 0.6–6.0) and 29% more

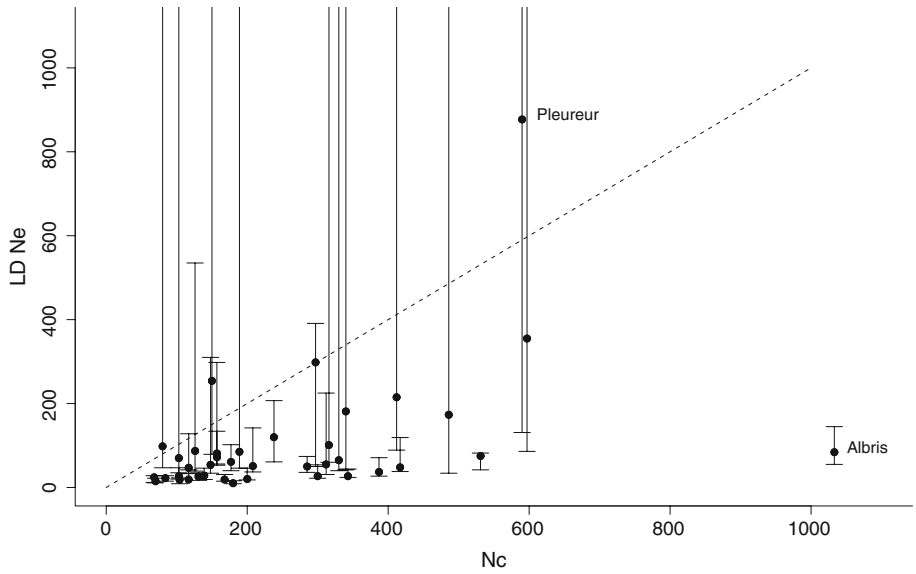

Fig. 3 Census size in 2007 (Nc) significantly influenced contemporary effective population size estimates obtained with the LD method (LD Ne; $b = 0.33 \pm 0.12$ SE; $F = 8$; $R^2 = 0.16$; $P = 0.007$). 95% confidence intervals of the LD Ne estimates are shown

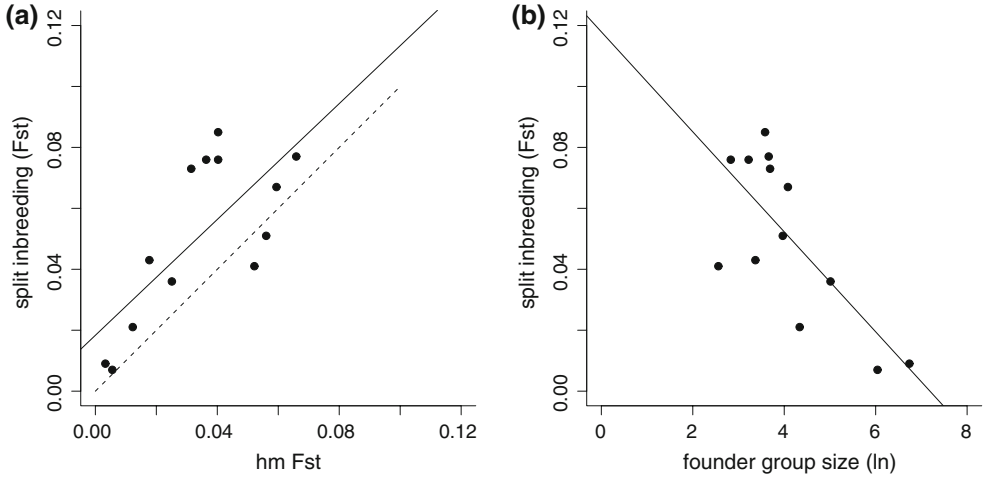

Fig. 4 Genetic estimates of inbreeding since the last split (split inbreeding) in relation to **a** harmonic mean population size since founding, transformed to Fst (hm Fst) and **b** to the founder group size (ln transformed). The solid lines correspond to the fitted linear regressions. The *dotted line* in **a** corresponds to the 1:1 ratio

inbreeding accumulated before compared to after the last split.

Discussion

Most conservation genetic studies of inbreeding use estimates of individual inbreeding coefficients to assess evidence for inbreeding depression within threatened populations. In the absence of pedigree information, obtaining estimates of individual inbreeding coefficients can be challenging (Allendorf and Luikart 2007). From a conservation perspective, inbreeding depression at the population level (e.g. reduced population growth rates) is

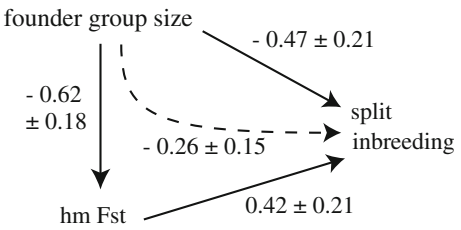

Fig. 5 Path analysis of the effects of founder group size (ln transformed) and harmonic mean population size (transformed to Fst) on split inbreeding. Solid lines represent direct effects and the dotted line the indirect effect. Numbers refer to the path coefficients ±SE. Note that the transformation of harmonic mean population size to Fst (hm Fst) changes the sign of the expected relationship with inbreeding

also crucial (Keller et al. 2007) and, thus, average inbreeding at the population level is of interest. Here we used population-specific Fst estimated from 37 microsatellite loci to assess population-level inbreeding in 41 reintroduced Alpine ibex populations. Because there is, on average, no deviation from Hardy–Weinberg proportions within these ibex populations (Fis ≈ 0, Biebach and Keller 2009), population-specific Fst measures total inbreeding in these populations (Vitalis et al. 2001; Holsinger and Weir 2009). Since population structure creates identity disequilibrium and thus correlation in heterozygosity across loci (Vitalis and Couvet 2001), these estimates of population-level inbreeding do not suffer from the same problems as individual inbreeding coefficients estimated from molecular data. Thus, when molecular data are available from a number of populations that are in Hardy–Weinberg proportion, population-specific Fst is a convenient way of measuring average inbreeding in a population. However, because Fst estimates are not only affected by statistical sampling variance but also by genetic sampling variance caused by genetic drift (Holsinger and Weir 2009), their confidence intervals are substantial even when based on three dozen loci (Table 1; Fig. 6).

We used two different reference populations to decompose total inbreeding into contributions from two phases of the reintroduction period. Together with population census data and estimates of the contemporary effective population size, we were able to identify the population parameters that contributed most to inbreeding in these reintroduced ibex populations.

Contemporary effective population size

We used samples collected more than 20 years ago to estimate contemporary Ne with the temporal method. These samples had on average 3.4-fold higher dropout and 5.5-fold higher false allele rates than the samples collected in 2004–2007 (Biebach and Keller 2009). The higher error rates are probably the consequence of repeated thawing and freezing and of radioactive radiation treatments following customs regulations when samples were shipped between laboratories (Scribner, "personal communication"). We omitted loci with high genotyping error rates from the estimation of Ne with the temporal method. These were thus based on fewer loci than the LD Ne estimates.

Estimates of contemporary Ne using the temporal and the LD method were not correlated across the eight populations for which we had estimates from both methods (Fig. 2), but Ne values were of similar magnitude. This is in line with a comparative study of Ne estimators (Fraser et al. 2007) where temporal and LD methods gave estimates that were uncorrelated but of similar magnitude. The ratio of LD Ne to temporal Ne of the ibex populations (mean: 1.18) was also similar to the ratio reported by Fraser et al. (2007) for isolated populations. Our Ne estimates might be biased downward, if there is undetected gene flow or admixture between populations, because both processes lead to an increase in linkage disequilibrium relative to the one caused by genetic drift alone (Nei and Li 1973; Fraser et al. 2007). In our study, possible source populations of undetected migrants have usually the same ancestral population as the recipient population. Thus, potential migrants are genetically similar (Biebach and Keller 2009) and any downward bias of Ne would thus be relatively small (Fraser et al. 2007). In addition to ongoing gene flow, admixture of the founder group might influence LD. However, LD decays at a rate of 0.5 per generation for unlinked loci**.** Since most ibex populations in this study were founded ca. 6 generations ago, only 1.6% of the LD among unlinked loci should be due to admixture of the founder group. Thus, a substantial downward bias in the Ne estimates due to ongoing gene flow or admixture is unlikely.

The mean LD Ne estimate of all 41 ibex populations (Ne = 102) suggests that, on average, ibex populations are currently loosing 0.5% of their expected heterozygosity per generation; for 17 populations this value was more than 1%. Note that Ne estimates from single populations should be taken with care because point estimates of Ne estimators are often inaccurate (Nei and Tajima 1981; Waples 1989; Fraser et al. 2007). This view is confirmed by the high confidence intervals of the Ne estimates and the non-correlation of the two temporal Ne methods in this study. However, the mean of Ne point estimates over several populations is more meaningful and thus may contain important information for conservation issues.

The mean Ne/Nc was 0.58 and thus somewhat higher than comparable values in other mammals: Frankham (1995) reported a mean of 0.35 in mammalian studies. However, this difference may be caused by a few unrealistic estimates of Ne in our data set where Ne exceeded the census size. Excluding these populations, we obtained a ratio (0.34) very similar to the one reported for other mammals (Frankham 1995). This ratio seems reasonable, given that Alpine ibex are highly polygynous, leading to a high variance in reproductive success of males and, in turn, to reduced Ne (Hoelzel 1999; Stiver et al. 2008). However, it is possible that the Ne/Nc ratio is biased upwards. We assumed that LD was influenced by genetic drift over the last four generations, and thus used the harmonic mean census size over the last four generations to calculate the Ne/Nc ratio. If genetic drift over less than the last four generations created the current LD, we would have overestimated the Ne/Nc ratio because some of the ibex populations are still growing and the harmonic mean population size is thus higher when calculated over less than four generations back. Moreover, although ibex censuses are relatively precise, they may consistently

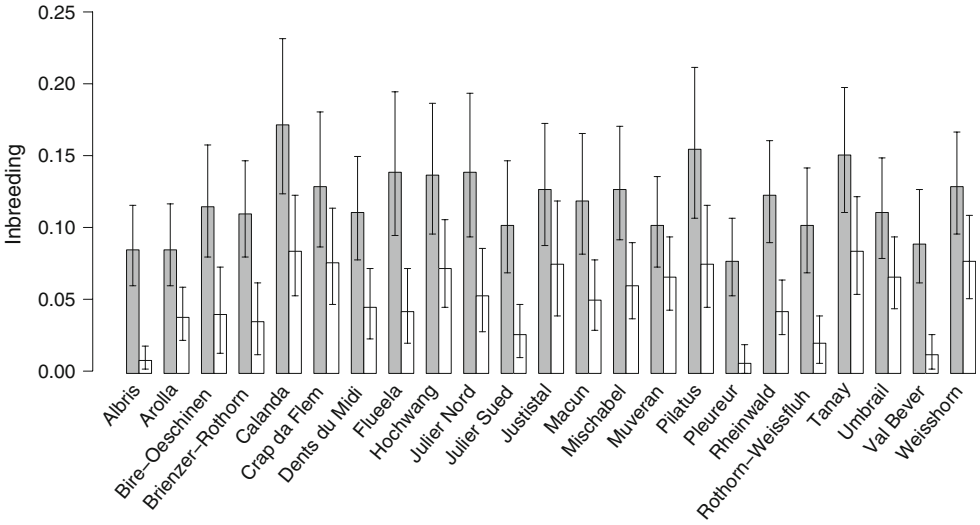

Fig. 6 Total inbreeding (*grey*) and inbreeding after the last split (*white*) for 23 reintroduced Alpine ibex populations. Error bars correspond to the 95% credible intervals of the inbreeding estimates (populations specific Fst)

underestimate actual population sizes (Saether et al. 2007). Such underestimates represent another cause of an upward bias in the Ne/Nc ratio.

Current census size and Ne were positively correlated (Fig. 3), suggesting that one could predict the effective population size from the census size. However, only 16% of the variation in Ne was explained by current census size. The low correlation between Ne and Nc was also reflected in the high range of Ne/Nc ratios among populations. In addition to variance introduced by the low precision of the Ne estimates (Nei and Tajima 1981; Waples 1989), other factors such as variance in family size or differences in sex ratio may also have contributed to the variation in Ne among populations. For example, some studies have reported an increased variance in reproductive success in larger populations (Ardren and Kapuscinski 2003; Hedrick 2005; Stiver et al. 2008). Additionally, hunting schemes and intensities differ among ibex populations, and this might affect Ne differently (Ryman et al. 1981; Allendorf et al. 2008). Overall, our results are consistent with several others that have shown that Ne/Nc ratios are often variable among populations within species (Waples 2002; Ardren and Kapuscinski 2003) and, therefore, cannot be assumed constant over time or space (Palstra and Ruzzante 2008).

Inbreeding during the two phases of the reintroduction program

Substantial inbreeding has accumulated in the ibex populations since the beginning of the reintroduction program. Mean total inbreeding was close to that expected from one generation of half sib mating. Note that this does not imply that half sib matings are taking place. Instead, this value reflects the accumulation of inbreeding over time in small populations even when matings between close relatives are rare (e.g. Ewing et al. 2008).

More inbreeding accumulated during the first than the second phase of the reintroduction program. In other words, of the total inbreeding that accumulated in populations that experienced two founder events, 59% accumulated after the first founder event and 41% after the second. This observation mirrors earlier results, which showed higher genetic differentiation between populations founded from the zoos and their sources than between populations founded later from wild populations and their sources (last split) (Biebach and Keller 2009). Thus, on average the earlier reintroductions lead to more inbreeding than later reintroductions. However, the ratio of inbreeding before to inbreeding after the last split was highly variable.

Some of the variation in inbreeding levels among ibex populations was caused by differences in founder group size and in harmonic mean population size (Figs. 4, 5). Harmonic mean population size (transformed to Fst) predicts the amount of inbreeding expected in populations of varying size. Since variance in reproductive success and other factors further reduced Ne, it is not surprising that populations were on average more inbred than expected from varying population size alone (Fig. 4). Founder group size had roughly the same direct effect on inbreeding as harmonic mean population size. However, founder group size had an additional indirect effect on inbreeding mediated by its effect on the harmonic mean population size. Thus, consistent with population genetic theory, less inbreeding resulted when populations were founded with more individuals and when they grew quickly to large population sizes. This finding reiterates the importance of fast growth after founding of populations in order to maintain genetic diversity (Nei et al. 1975) and reduce inbreeding.

Contemporary effective population size did not explain a significant proportion of the variation in total inbreeding. Thus, estimates of the current effective population size did not reflect overall levels of inbreeding in these reintroduced

populations. This is not surprising given that both of the Ne estimators we employed here do not cover the early periods of population growth following the reintroductions: The temporal Ne estimates cover the period 1986–2007 and the LD Ne estimates cover the last 3–5 generations, i.e. approximately the last 24–40 years. Note also, that temporal Ne and LD Ne estimate the variance effective population size, while inbreeding is related to the inbreeding effective size (Crow and Kimura 1970, pp. 345–364). However, we expect the error introduced by calculating variance instead of inbreeding effective size to be small compared to the error of calculating Ne and inbreeding over two very different time periods.

Conclusion

The effective population size (Ne) is an important tool in the management of threatened species and captive populations (Leberg 2005). However, as emphasized by our results from 41 reintroduced Alpine ibex populations, contemporary Ne does not reflect average levels of inbreeding in reintroduced populations that, by definition, have varied greatly in size in their recent past. Thus, independent estimates of population-level inbreeding are an important addition to estimates of contemporary Ne in the genetic management of reintroduced populations. Alternatively, inference of historical Ne may yield similar information (Leberg 2005).

Minimizing inbreeding is a central goal of conservation genetic management. In reintroduction programs, this might be achieved in two different phases of the reintroductions: First, releasing a large number of founders can reduce inbreeding. Second, fast population growth following the founder event increases the harmonic mean population size and thus reduces inbreeding. Therefore, population genetic theory (Jacquard 1974, chap 8) and our results suggest that boosting population growth rate with further releases will help reduce inbreeding even when, from a purely demographic point of view, no further releases are necessary to yield a self-growing population (Schaub et al. 2009).

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Appendix

See Table 2.

Table 2 Estimated genotyping error rates for samples of the first sampling period (1986–1988)

Locus	Dropout	False
BM1225[a]	0.000	0.046
BM2113	0.048	0.000
BM302	0.000	0.000
BM415	0.024	0.000
BM4505[a]	0.090	0.000
CSSM47	0.000	0.004
HAUT27[a]	0.127	0.044
IDVGA30	0.000	0.008
ILSTS29	0.031	0.000
ILSTS30	0.034	0.000
INRABERN172	0.020	0.000
INRABERN175[a]	0.106	0.000
INRABERN185[a]	0.081	0.053
JMP29	0.017	0.000
MAF209	0.000	0.000
MAF36	0.000	0.000
MAF70	0.000	0.000
McM152	0.000	0.000
McM173	0.000	0.000
MILSTS076	0.046	0.000
OarAE54	0.030	0.000
OarFCB193	0.000	0.000
OarFCB20	0.000	0.000
OarFCB48	0.000	0.000
OARHH35	0.012	0.000
OarVH34	0.050	0.000
SR-CRSP01	0.019	0.000
SR-CRSP08[a]	0.238	0.046
SR-CRSP09	0.034	0.000
SR-CRSP23	0.036	0.000
SR-CRSP24	0.049	0.000
SR-CRSP25	0.024	0.000
TGLA10	0.009	0.000
TGLA122	0.047	0.000
TGLA126	0.000	0.000
TGLA73	0.000	0.000
URB058[a]	0.071	0.000
Mean	0.034	0.005

Dropout: allelic dropout rate per heterozygote; False: false allele rate per genotype

[a] Loci omitted from the Ne estimation with the temporal method

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